RESEARCH REPORT
SWINE HEALTH

Title: Development of stable cell lines permissive for PRRSV replication and production
NPB #06-145

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INDUSTRY SUMMARY:

PRRS virus (PRRSV) infects only a specific line of simian cells in culture for productive infection. The use of these cells for vaccine development is patent-protected, which severely hampers the development of any new vaccine for PRRS. The recent development of infectious clones make it possible to construct a genetically engineered PRRSV vaccine candidate, but even in this case the engineered virus needs to be propagated in cell culture and therefore an appropriate cell line permissive for PRRSV devoid of patent protection is an essential requirement for development of a new vaccine. In the present study, stable cell lines permissive for PRRS virus were developed. The gene for CD163, a recently described cellular receptor for PRRSV, was cloned and introduced into several different lines of porcine kidney cells which were naturally non-permissive for PRRSV. These cells expressed CD163 on the cell surface, and became permissive for PRRSV and produced infectious virus. These cells are additional reagents for PRRSV research and also may serve as an alternative source for PRRSV production. The newly developed PRRSV permissive cells are freely available to any researchers in the PRRS community.
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III. SCIENTIFIC ABSTRACT:

Virus infection is initiated by interaction of the viral ligand with the viral-specific cellular receptor on the cell surface. For PRRSV, at least four putative receptor molecules have been described; sialoadhesin, vimentin, CD151, and CD163. We cloned the CD163 gene from porcine macrophages and introduced into cells that are PRRSV non-permissive. The stable expression of pCD163 was confirmed by RT-PCR, immunofluorescence, and Western blot assays. When these cells were infected with PRRSV, they became fully permissive for PRRSV infection and produced infectious virus. Crandall feline kidney (CRFK) cells however remained non-permissive despite the CD163 expression was evident. Transfection of CRFK cells with an infectious clone of PRRSV produced infectious progeny in these cells, suggesting the interruption of infection in CRFK cells was associated with the virus entry. When CRFK cells were co-expressed with pCD163 and vimentin followed by infection with PRRSV, the cells became permissive for PRRSV and infectious virus was produced. Our study suggests that CD163 is a major determinant for PRRSV infection but requires a co-factor for permissiveness of cells.

IV. INTRODUCTION:

1) Production of commercial vaccines and PRRSV permissive cell systems: PRRSV exhibits highly restricted host specificity *in vivo* and cell tropism *in vitro*. PRRSV replicates in porcine monocytes and macrophages, especially in porcine alveolar macrophages (PAMs), and to a lesser extent in testicular germ cells such as spermatides and spermatocytes in boars. *In vitro,* PRRSV replicates in primary cultures of PAMs, as well as in the specific line of African green monkey kidney cells such as CL-2621 or MA-104 and its derivative MARC-145 cells. A modified live virus vaccine has been commercialized by Boehringer Ingelheim (BI), and this is the most commonly used vaccine for PRRS in the US. The BI vaccine was developed through the multiple passages of virus in monkey kidney cells, and this vaccine confers appropriate levels of protective immunity for homologous virus infection, but heterologous protection seems to be limited. Inactivated vaccines show minimal levels of protection. A major concern associated with the current BI vaccine is its safety. The BI vaccine has a potential to revert to virulence in pigs upon vaccination, and thus concerns are raised among veterinarians and researchers. Notably, the BI vaccine-associated outbreaks have been reported in Denmark. The BI vaccine persists in the vaccinated pigs for weeks and can spread to naïve animals by normal transmission or by semen. The vaccine virus also crosses the placenta and causes congenital infection in fetuses. A consensus is the need for a better vaccine for PRRSV.

Attempts have been made to identify cell lines that are permissive for PRRSV replication in culture. However, a specific line of simian kidney cells is currently the only identified established cell line supporting PRRSV replication, and the use of these cells for commercial purpose is patent-protected. This patent is valid for 20 years and therefore severely hampers the development of any new PRRS vaccine by other investigators until the patent rights expire. The lack of an appropriate cell line, free from patent protection, to support the growth of PRRSV is the prime obstacle for development of a new vaccine at the present time. The recent development of infectious cDNA clones for PRRSV and the available reverse genetics system allow us to manipulate the viral genome and construct genetically engineered mutant PRRSV. Genetically engineered PRRSV may be safer and more efficacious with an appropriate genetic marker that can be used for differentiation from field virus. Even in this case however, the constructed vaccine virus from the infectious cDNA clone still needs to be propagated in cell culture, and thus an appropriate cell line permissive for PRRSV replication is essential for new vaccine development.

2) CD163 as a PRRSV-specific cellular receptor and the development of cell lines permissive for PRRSV: Among factors contributing to permissiveness of cells for virus infection, the presence or absence of a specific receptor is one of the major determinants. PRRSV enters cells by receptor-mediated endocytosis, but the identity of cellular receptors for PRRSV entry is complicated by various reports. Recently, CD163 has been suggested as a putative receptor for PRRS virus. CD163 is a membrane-associated glycoprotein expressed on the cell surface of macrophages. The cell-specific expression of CD163 is consistent with macrophage lineage-specific infection of PRRSV, with an exception of Marc-145 cells. CD163 is a hemoglobin scavenger receptor (HbSR), belonging to the group B cysteine-rich scavenger receptor (SRCR) superfamily. The objective of this study was to develop PRRSV permissive stable cell lines by expression of CD163 in non-permissive cells, so that these cells may be used as an alternative source for PRRSV production. These cells will also be useful as a tool for PRRSV research since the Marc-145 cells widely used for PRRSV research are originated from a monkey which PRRS does not naturally infect.

V. OBJECTIVES:

Objective 1: To determine the function of porcine CD163 expression as the PRRSV permissive factor

Objective 2: To develop cell lines stably expressing CD163 so that they become permissive for PRRSV and may be used for production of PRRSV and pathogenesis studies.

VI. MATERIALS & METHODS:

Cells, Virus, and antibodies: Marc-145 cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT) in a humidified incubator at 37C with 5% CO2. LLC-PK cells were maintained in minimum essential medium

(MEM) with 5% FBS. Dulac cells were cultivated in minimum essential medium (MEM) supplemented with 10 % (v/v) FBS. Crandall feline kidney (CRFK) cells were maintained in MEM with 5% FBS. ΔC2, monocyte-derived porcine macrophages, were obtained from Dr. Chitko-Mckown at USDA (Clay Center, NE) and maintained in RPMI with 10% FBS. Dulac-pCD163 cells were maintained in the presence of 800 μg/ml of G418 (GIBCO/BRL, Gaithersburg, MD). For PRRSV, the PA8 strain of the North American type was used throughout the study. Mouse monoclonal antibodies (MAb), 2A100/11, specific for porcine CD163 were purchased from AbD Serotec (Raleigh, NC). Goat anti-mouse immunoglobulin IgG-conjugated to Alexa 488 dye (Molecular Probes, Eugene, OR) was used for immunofluorescence assay and goat anti-mouse immunoglobulin IgG-conjugated to horseradish peroxide (HRP) (Jackson ImmunoResearch Laboratories, West Grove, PA) was used for Western blot analysis.

DNA transfection and selection of G418 resistant cells: Transfection was carried out using the Amaxa Nucleofector transfection system (Amaxa, Gaithersburg, MD) according to the manufacturer's protocol. Fresh cells were grown to confluence 2 days prior to DNA transfection. Cells were trypsinized and approximately 2 million cells were transfected with 2 μg of DNA using Amaxa and plated in a 100-mm diameter dish. At 24 hr post-transfection, geneticin (G418 sulfate) (Gibco/BRL) selective antibiotic was added to complete medium to a final concentration of 1 mg/ml. At four days after addition of the G418 selective medium, most cells were dead and only a few cells remained alive. The medium was then replaced with fresh complete medium containing G418 at 1 mg/ml. These few healthy alive cells were further cultured with selective medium which was changed with fresh one every 4 days. After 3-4 weeks cultivation, the visible individual colonies were formed and ready for cloned out.

RNA extraction, DNase treatment, and RT-PCR: Total cellular RNA was extracted from cells using Trizol (Invitrogen). The total RNA was subjected to DNase treatment (RQ DNase I, Promega), followed by phenol chloroform extraction and ethanol precipitation. Reverse transcription (RT) reaction was carried out with a 25 μl reaction mixture containing 12 μl RNA, 3 μl reverse primer, 5 μl 5x buffer, 1.5 μl dNTP mix (10 mM), 2 μl DTT (10 mM), 0. μl RNase inhibitor, 0.5 μl RNase inhibitor and 1 μl M-MLV reverse transcriptase (200U/μl, Invitrogen). The reverse transcriptase step was carried out at 42 °C for 1 hr and 95 °C for 5 min, followed by 30 cycles of amplification (94 °C for 30 s; 56 °C for 30 s; 72 °C for 30 s). Viral RNA was extracted from cell culture supernatants using QIAamp Viral RNA kit (QIAgen). For PCR amplification, the upstream (5'-CGATCATGCTGAGGATGCTGGAG-3') and downstream primers (5'-AGAGAGCAGACTCGTGTCCATGGC-3') were used for detection of pCD163 gene. ORF7-Fwd and ORF7-Rv were used for N gene detection. PCR was performed by 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 sec, 56 °C for 30 sec and 72 °C for 30 sec, and finalized by extension at 72 °C for 10 min.

Immunofluorescence assay (IFA): The cells were seeded on microscope coverslips placed in 35-mm dishes and grown to confluency. Cells were then washed once with PBS and fixed with 4% paraformaldehyde for 10 min. After blocking with 1% bovine serum albumin (BSA) for 30 min at room temperature (RT), the cells were permeabilized with 0.1% NP-40 for 10 min at RT. Cells were then incubated with pCD163-specific MAb 2A100/11 for 1 hr at RT followed by incubation with Alexa Fluor 488 conjugated goat anti-mouse secondary antibody (Molecular Probes) for 1 hr at RT. Cells were washed 3 times with PBS and the coverslips were mounted on microscope slides using 60% glycerol in PBS containing 0.1% Na-azide. pCD163 expression was visualized under a fluorescence microscope. PRRSV P129-GFP infected cells were visualized directly by fluorescence microscopy at 5 days post-infection.

SDS-PAGE and Western blot: Fresh cells were grown to confluence in complete medium. Cells were then lysed in lysis buffer (20 mM Tris [pH7.5], 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1% NP40) for 30 min on ice. After centrifuged at 13,000 rpm for 20 min, the cell lysates were collected and boiled for 5 min in 2 × sample buffer (100 mM Tris-HCl [PH7.5], 4% [w/v] SDS, 0.2% [w/v] bromophenol blue, 30% [w/v] glycerol). The proteins were analyzed by electrophoresis on 7.5% SDS-PAGE gels. Proteins were then

transferred to a PVDF membrane (Millipore). The membrane was blocked using 5 % (w/v) of skim milk in TBST (10 mM Tris [PH8.0], 150 mM NaCl, 0.1% Tween 20) overnight. The membrane was then reacted with porcine CD163 MAb 2A100/11 for 1 hr at room temperature followed by reacting with HRP conjugated goat anti-mouse IgG secondary antibody for another 1 hr at room temperature. The presence of biotinylated proteins was detected by chemiluminescence detection system according to the manufacturer's suggestions (Pierce).

Plaque assay: Marc-145 cells were seeded in 6-well plates. For each sample, at least 2 sets of cells were prepared for duplication. Cell culture supernatants at 24 hr and 48 hr post-infection were serially diluted in MEM and the virus titers were determined by standard plaque assay. Cells were stained at 5 days with crystal violet and calculated as PFU (plaque forming unit) per ml.

VII. RESULTS:

Cloning and sequencing of porcine CD163 genes: To make this project successful, two questions needed to be addressed; 1) Is CD163 a cellular receptor for PRRSV and 2) Does CD163 confer permissiveness of cells for PRRSV. As the first step to address those questions, we cloned the gene for CD163 from pigs. Using information available in the public database, the full-length cDNA for porcine CD163 (pCD163) was cloned from porcine alveolar macrophages (PAMs) and sequenced subsequently. The full-length gene for porcine CD163 was 3,345 nucleotides, coding for a protein of 1,115 amino acids (Fig. 1). It was slightly smaller than human CD163 which was 3,471 nucleotides for 1,157 amino acids. CD163 is a type I membrane protein containing the transmembrane anchor domain and is expected to be expressed on the cell surface and to function for virus attachment.

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MDKLRMVLHE NSGSADFRRC SAHLSSFTFA VVAVLSACLV TSSLGGKDKE LRLTGGENKC SGRVEVKVQE EWGTVCNNGW
DMDVVSVVCR QLGCPTAIKA TGWANFSAGS GRIWMDHVSC RGNESALWDC KHDGWGKHNC THQQDAGVTC SDGSDLEMGL
VNGGNRCLGR IEVKFQGRWG TVCDDNFNIN HASVVCKQLE CGSAVSFSGS ANFGEGSGPI WFDDLVCNGN ESALWNCKHE
GWGKHNCDHA EDAGVICLNG ADLKLRVVDG VTECSGRLEV KFQGEWGTIC DDGWDSDDAA VACKQLGCPT AVTAIGRVNA
SEGTGHIWLD SVSCHGHESA LWQCRHHEWG KHYCNHDEDA GVTCSDGSDL ELRLKGGGSH CAGTVEVEIQ KLVGKVCDRS
WGLKEADVVC RQLGCGSALK TSYQVYSKTK ATNTWLFVSS CNGNETSLWD CKNWQWGGLS CDHYDEAKIT CSAHRKPRLV
GGDIPCSGRV EVQHGDTWGT VCDSDFSLEA ASVLCRELQC GTVVSLLGGA HFGEGSGQIW AEEFQCEGHE SHLSLCPVAP
RPDGTCSHSR DVGVVCSRYT QIRLVNGKTP CEGRVELNIL GSWGSLCNSH WDMEDAHVLC QQLKCGVALS IPGGAPFGKG
SEQVWRHMFH CTGTEKHMGD CSVTALGASL CSSGQVASVI CSGNQSQTLS PCNSSSSDPS SSIISEENGV ACIGSGQLRL
VDGGGRCAGR VEVYHEGSWG TICDDSWDLN DAHVVCKQLS CGWAINATGS AHFGEGTGPI WLDEINCNGK ESHIWQCHSH
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KTVSRHMWVD NVQCPKGPDT LWQCPSSPWK KRLASPSEET WITCANKIRL QEGNTNCSGR VEIWYGGSWG TVCDDSWDLE
DAQVVCRQLG CGSALEAGKE AAFGQGTGPI WLNEVKCKGN ETSLWDCPAR SWGHSDCGHK EDAAVTCSEI AKSRESLHAT
GRSSFVALAI FGVILLACLI AFLIWTQKRR QRQRLSVFSG GENSVHQIQY REMNSCLKAD ETDMLNPSGD HSEVQ
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Fig. 1. Amino acid sequence of porcine CD163. Porcine CD163 is a type I membrane protein of 1,355 amino acids in size.

To determine the role of pCD163 as the permissive factor for PRRSV, the CD163 gene was inserted into a mammalian expression vector and used for transient expression in Dulac porcine kidney cells by DNA transfection. Dulac cells were naturally non-permissive for PRRSV infection, and therefore these cells were ideal for permissiveness studies. Dulac cells were transfected with CD163 and then infected with PRRSV. The cells became permissive for PRRSV replication after transfection. The titer of PRRSV in these cells was relatively low, but infection was clearly positive. The low level replication of PRRV was not unexpected since only a small fraction of cells would express the gene by transient transfection and so the level of CD163 expression would be limited. Nevertheless, this experiment showed that CD163 could convert non-permissive cells to permissive. A recent report also supports our finding that CD163 is the cellular receptor for PRRSV and is the permissive factor.

Establishment of a cell line stably expressing pCD163: Dulac cells were transfected with pCD163. G418 was added to the media and cells were further incubated until G418-resistant cells grew to form colonies. Individual cell colonies were picked and transferred to fresh plates for amplification. To determine the CD163 gene expression in these cells, RT-PCR was performed (Fig. 2). A 284 bp fragment was specifically amplified from

these cells, indicating that these cells synthesize specific mRNA for CD163. To determine the CD163 protein in these cells, IFA was conducted using a specific MAb for pCD163 (Fig. 3). Fluorescence signal was evident whereas untransfected cells remained negative. Western blot showed a specific band of 120 kD protein, confirming CD163 expression (Fig. 4). Taken together, the G418-selected Dulac cells stably expressed pCD163. These cells were designated Dulac-pCD163.

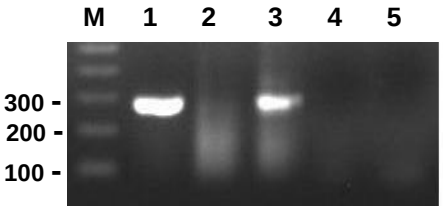

Fig. 2. RT-PCR for pCD163 in Dulac-pCD163 cells. RNA was extracted and treated with DNase followed by RT-PCR (lanes 3 and 5) or PCR (lanes 2 and 4). Lane 1, a positive control in which pCD163 DNA was used as a template for PCR; Lanes 2, 3, Dulac-pCD163 cells without and with DNase treatment, respectively; Lane 4, 5, parental Dulac cells without and with DNase treatment, respectively.

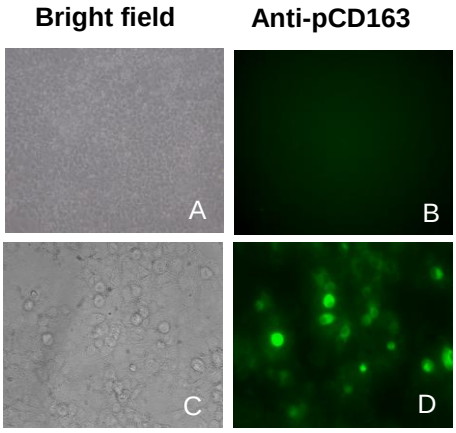

Fig. 3 Porcine CD163 expression in Dulac-pCD163 cells. Cells were fixed and incubated with the porcine specific CD163 MAb for immunofluorescence. A, B, bright field; C, D, fluorescence.

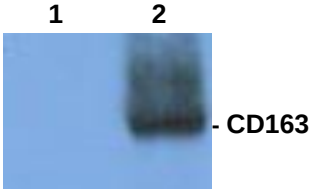

Fig. 4. Expression of pCD163 in Dulac-pCD163 cells. Cells were lysed and the lysates were resolved by 7.5% SDS-PAGE under non-reducing conditions without βME. Proteins were transferred to a PVDF membrane and reacted with porcine CD163 MAb. Lane 1, Dulac cells; lane 2, Dulac-pCD163 cells.

PRRSV infection in Dulac-pCD163 cells: Dulac-pCD163 cells were next examined for their permissiveness for PRRSV infection. Cells were infected with PRRSV expressing green fluorescence protein (GFP). After 1 hr infection, cell monolayer was washed three times with PBS, and was incubated for 1 week at 37˚C. By day 5 post-infection, Dulac-pCD163 cells showed green signal under fluorescent microscopy, indicating that PRRSV was replicating in these cells and produced the GFP protein suggesting the permissiveness of the cells for PRRSV (Fig. 5). At further incubation, the monolayer showed cell rounding, clumping and sloughing off. The morphological changes in these cells upon infection were similar to the cytopathic effects seen in PRRSV-infected MARC-145 cells.

To confirm the production of PRRSV from these cells, RT-PCR was performed to detect N gene from the culture supernatant. A 400 bp product was specifically amplified from PRRSV-infected Dulac-pCD163 cells (data not shown), and the sequencing confirmed the presence of the viral gene in the supernatant. To further determine the infectivity in the culture supernatant, fresh MARC-145 cells were inoculated with the supernatant. By 5 days post-infection, GFP-induced fluorescence was evident (Fig. 6), indicating the presence of infectivity in the supernatant obtained from Dulac-pCD163. The titer of virus reached $3x10^3$ PFU/ml, and after 3 consecutive passages of the virus in Dulac-pCD163, increased to 10^5 PFU/ml which is the level similar to titers in MARC-145 cells. Another line of cells (LLC-PK porcine kidney cells) was established to express pCD163,

and these cells were named LLCPK-pCD163. LLCPK-pCD163 cells were permissive to PRRSV upon CD163 expression (data not shown), and the virus production in these cells was similar to that in Dulac-pCD163.

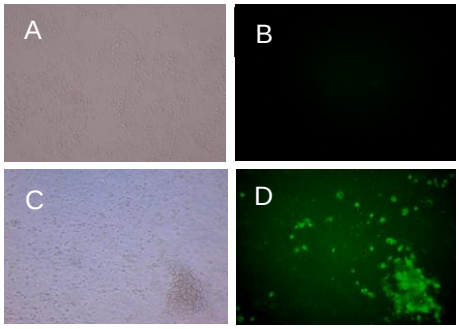

Fig. 5. Infection and production of PRRSV in Dulac-pCD163 cells expressing pCD163. Cells were infected with PRRSV-GFP virus expressing green fluorescence protein. At 1 week of infection, cells were monitored for GFP expression

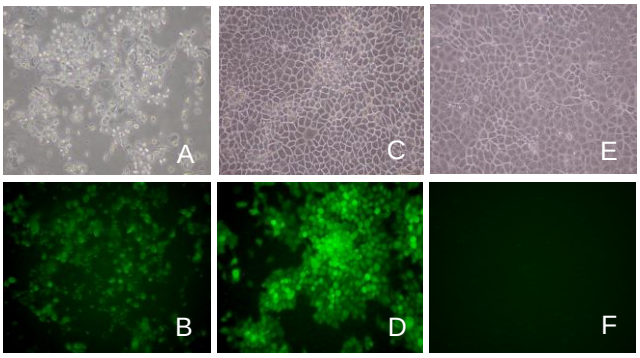

Fig. 6. Production of PRRSV from Dulac-pCD163 cells. Supernatants were collected from Dulac-pCD163 cells 7 days post-infection with PRRSV-GFP and used to inoculate Marc-145 cells. Panels: A,B, Marc-145 cells infected with PRRSV-GFP; C,D, Marc-145 cells infected with the supernatant collected from PRRSV-GFP-infected Dulac-pCD163; E,F, Dulac cells infected with PRRSV-GFP.

PRRSV infection in CRFK-pCD163 cells and the role of vimentin: Besides LLCPK-pCD163 and Dulac-pCD163 cells, CRFK-pCD163 cells were also made to express pCD163. CRFK-pCD163 cells exhibited high level expression of pCD163 (Fig. 7), shown by RT-PCR, IFA, and immunoblot. However, CRFK-pCD163 cells remained non-permissive for PRRSV and did not support PRRSV replication. While other cell types were converted to be permissive for PRRSV when expressing CD163, CRFK cells were not converted to allow infection even when CD163 expression was high. This finding suggests that it is possible that, in addition to CD163 as the major receptor, a co-receptor may be required in the PRRSV cell entry, and the co-receptor is absent in CRFK cells. Previous reports suggested vimentin, CD151, and sialoadhesin as a possible receptor for PRRSV, but none of these molecules alone was able to convert non-permissive cells to permissive. Since CD163 alone can convert other cell types to permissive, except CRFK cells, we expanded our study to determine if any of those molecules was indeed required for PRRSV cell entry in CRFK cells.

To determine if the blocking of infection occurred at the level of virus-cell receptor interactions or during the intracellular process of virus replication, CRFK cells were transfected with an infectious clone of PRRSV. Transfection with an infectious clone bypasses receptor interactions for virus replication. After transfection, infectious virus was obtainable from the culture supernatant, demonstrating the CRFK cells support the PRRSV replication. This result suggests that the non-permissiveness of CRFK-pCD163 cells is at the entry step.

Since vimentin was shown to be a possible co-receptor for PRRSV, we examined the presence of vimentin in various cell types (Fig. 8). The vimentin expression was high in MARC-145 cells, Dulac cells, and LLCPK cells. Interestingly, CRFK cells showed only a low level of vimentin expression, supporting out hypothesis that a co-receptor is required for PRRSV and that vimentin may serve as the co-receptor. To test this hypothesis, CRFK-pCD163 cells were transfected with the vimentin gene to allow co-expression of pCD163 and vimentin, followed by infection with PRRSV. As expected, infectious PRRSV was produced from CRFK cells co-expressing pCD163 and vimentin, demonstrating that vimentin served as a co-factor for PRRSV permissiveness along with pCD163.

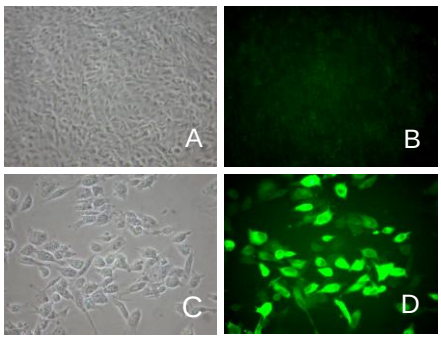

Fig. 7. Expression of porcine CD163 from stably transfected CRFK cells. Cells were stained with the CD163 specific monoclonal antibody 2A10/11, followed by staining with the fluorescent conjugated goat anti-mouse secondary antibody. Panels: A, C, bright fields; B, D, fluorescent fields.

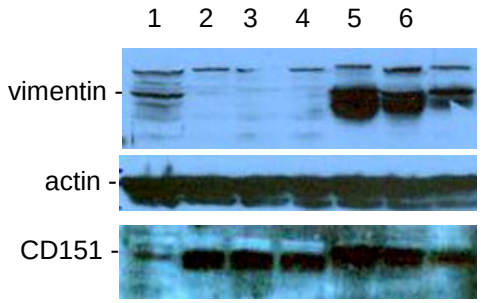

Fig. 8. Minimal expression of vimentin in various cells. Cell lysates were resolved by SDS-PAGE and transferred to PVDF membrane, followed by Western blot using the vimentin specific Mab. Vimentin, multiple forms at around 57 kDa; CD151, 28-32 kDa; β-actin: 43 kDa in size. lanes,: 1, Marc-145 cells; 2, CRFK; 3 and 4, CRFK-pCD163; 5, Dulac; 6, Dulac-pCD163; 7,ΔC2

VIII. DISCUSSION:

MARC-145 cells are permissive for PRRSV and are the only continuous line of cells in culture for productive infection of PRRSV. These cells are widely used in the laboratories for PRRSV research. However, MARC-145 cells are of simian origin, and questions remain as for the use of simian cells for porcine virus for cell-virus interaction studies. Furthermore, the use of these cells for vaccine production is patent-protected and hampers the development of a new vaccine. In the present study, several lines of cells were developed to stably express porcine CD163. The PRRSV-non-permissive Dulac and LLC-PK cells were transfected with the CD163 gene and stably expressing cells were established. Upon expression of CD163, these cells became permissive for PRRSV and produced infectious virus. These cells may be used as an alternative source for PRRSV production and the study of cell-PRRSV interactions. These newly developed PRRSV permissive cells are freely available to any researchers in the PRRS community. During the course of our study, we found that CRFK cells did not become PRRSV-permissive even in the presence of CD163. Interestingly, vimentin expression in CRFK cells was minimal or undetectable. These cells however were converted to support PRRSV infection when vimentin was co-expressed with CD163. This result indicates the role of vimentin as a co-factor for PRRSV permissiveness.

CD163 plays an important role in maintaining host homeostasis and host immune regulation. It acts as an endocytic receptor of hemoglobin:haptoglobin (Hb-Hp) complexes. Hemoglobin released in the plasma is captured by haptoglobin to form Hb-Hp complexes. The Hb-Hp complexes then bind to CD163 expressed on the plasma membrane of macrophages, and mediates the clearance of hemoglobin by macrophages. This prevents the accumulation of Hb in the plasma and associated toxicity of the ion-containing heme molecule. CD163 is a type I membrane glycoprotein with an extracellular region, consisting of 9 repeats of the SRCR domain, and a transmembrane domain, followed by a short cytoplasmic tail at the C-terminus. CD163 expression is tightly regulated by a variety of factors. Differentiation of monocytes *in vitro* to macrophages promotes CD163 expression. It is also upregulated by glucocorticoids as well as anti-inflammatory mediators such as IL-6 and IL-10. On the contrary, pro-inflammatory mediators such as LPS, IFN-γ, and TNF-α suppress CD163 expression. In summary, we have successfully developed several lines of cells permissive for PRRS infection. These cells are useful for research on PRRSV-cell interaction and may serve as a source of PRRSV

production. Cell stocks were made for distribution upon request from other investigators, and these cells are freely available to any interested researchers in the PRRSV community.

IX. DISEMINATION OF THE RESULTS FROM CURRENT RESEARCH

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2) Song C., K. Chang, C. G. Chitko-McKown, D. Bienzle, F. A., Zuckermann, and D. Yoo. 2007. The role of CD163 for PRRSV infection. 88th Conference of Research Workers in Animal Disease (CRWAD), Chicago IL. Dec 2-4.
3) Song C., K. Chang, C. G. Chitko-McKown, D. Bienzle, F. A., Zuckermann, and D. Yoo. 2007. The role of CD163 for PRRSV infection. International PRRS Symposium. Chicago, IL. Nov 30-Dec 1.
4) Patton, J. B., R. R. Rowland, d. Yoo, and K. O. Chang. 2007. Replication of PRRS virus in association with CD163. International PRRS Symposium. Chicago, IL. Nov 30-Dec 1.